is limited understanding on the mode of action of glycans in RA. We hypothesise that the glycans on ACPA interact with glycan binding receptors and thus modulate immune responses in RA. Therefore, our aim is to elucidate the glycan effect of ACPA and other glycans on immune cells of RA patients to increase our understanding of RA pathogenesis.

Methods A whole blood flow assay was used to study glycan interactions with leukocytes. Leukocytes were isolated from blood and cells were incubated for 2 hours at 4°C with 15 µg/ml highly glycosylated ACPA and immunomodulatory glycoconjugates, such as sialic acid, Lewis-x, Lewis-y, mannose and as a negative control GlcNac. Glycan binding and identification of immune cell subsets was assessed with flow cytometry using a whole blood flow antibody panel.

Results This study examined the glycan-binding capacity of leukocytes in healthy donors via the whole blood flow assay. B cells appear to be superior in their interaction capacity with a variety of glycans, including the Fab glycan on APCA. This is an important finding because B cells play a key role in the pathogenesis of RA, through their antigen presenting capacity as well as their production of ACPA. In future studies RA patients material will be used to assess the glycan binding capacity to immune cell and to elucidate their role in the pathogenesis of RA.

Conclusions This study examined the glycan-binding capacity of leukocytes in healthy donors via the whole blood flow assay. B cells appear to be superior in their interaction capacity with a variety of glycans, including the Fab glycan on APCA. This is an important finding because B cells play a key role in the pathogenesis of RA, through their antigen presenting capacity as well as their production of ACPA. In future studies RA patients material will be used to assess the glycan binding capacity to immune cell and to elucidate their role in the pathogenesis of RA.

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P003 IGF1R SIGNALLING IS ESSENTIAL FOR NEUROLOGICAL SYMPTOMS IN RA

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Introduction In addition to inflammation of the joints, rheumatoid arthritis (RA) has a neurological part consisting of pain, fatigue, depression and cognitive deterioration. These symptoms are critical for the patients' ability to cope with daily life, but are not alleviated completely with modern anti-rheumatic drugs. Sufficient IGF1R signalling is important for neurogenesis in the hippocampus. Its misbalance correlates with depression and anxiety.

Objectives The aim of this study was to evaluate the pathological changes in the brain during experimental RA and investigate their connexion to IGF1R.

Methods Characteristics of pain, depression and anxiety were collected among 214 RA patients and analysed in relation to IGF1R expression in WBC. Experimental RA was induced by immunisation with collagen II. Inhibition of IGF1R was achieved by injection of shRNA-producing lentiviral construct. The behavioural pattern of each mouse was recorded by filming. The hippocampus was analysed morphometrically, and gene and protein expression were analysed by qPCR and immunohistochemistry, respectively.

Results The RA patients' perception of depression and anxiety was associated with high IGF1R expression in WBC. This group of patients was also less physically active. In experimental RA, an enrichment of IBA1 +microglia and high expression of CD68 and IL-1b was found in the hippocampus. This was followed by an increased density of IGF1R+cells in cornu ammoni, and a decreased neurogenesis by limited expression DCX in the subgranular layer of the dentate gyrus. This results in a significant reduction of the hippocampus area. These changes in the brain were associated with immobility in RA mice. Treatment with shRNA targeting IGF1R improved arthritis, but led to increased immobility.

Conclusions RA induces remote inflammation in the hippocampus reducing neurogenesis and physical activity. The neurological symptoms in patients and in experimental RA are connected to IGF1R expression and signalling, and further expands our knowledge of neurological processes in RA.

Disclosure of interest None declared

P004 ABSTRACT WITHDRAWN

P005 INFLUENCE OF MACROPHAGE POLARISATION ON EXPRESSION OF PEPTIDYLARGININE DEIMINASES 2 AND 4 THAT CATALYSE CITRULLINATION OF THE PROTEINS TARGETED BY ANTI-CITRULLINATED PROTEIN/PEPTIDE AUTOANTIBODIES (ACPA)

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Introduction Autoantibodies to citrullinated proteins (ACPA) are specifically associated to rheumatoid arthritis (RA) and probably involved in its pathophysiology. ACPA are produced in the inflamed synovial tissue (ST). We demonstrated that peptidylarginine deiminases (PAD) 2 and 4 are present in the tissue and probably responsible for fibrin citrullination, and consequently for genesis of the epitopes targeted by ACPA. PAD2 and 4 are expressed in the intima and the subintimal inflammatory infiltrates, essentially by CD68 +mononuclear cells.[1] We suspected macrophages (MΦ) of the ST to be responsible for synthesis and release of PADs in the interstitium, inducing citrullination of the local fibrin deposits. We generated *in vitro* various subsets of MΦ in the presence of IFN-γ, IL-4, IL-10 or M-CSF, and observed a major influence of the phenotype of polarised MΦ on cytokine response to ACPA-containing immune complexes.[2]

Objectives The aim of the current study was to evaluate expression of PAD2 and 4 by various subsets of polarised MΦ.

Methods CD14-positive monocytes from healthy donors were cultured in presence of M-CSF, IFN-γ, IL-4 or IL-10. The expression of the *PADI*2 and 4 genes was measured by RT-qPCR and the expression of PAD2 and 4, evaluated by immunoblotting of total cell extracts.
Results *PADI*2 and 4 mRNAs are expressed in monocytes while PAD2 and 4 proteins are also immunodetected in these cells. In the different MΦ subsets, *PADI*2 mRNAs are less detected than in the related monocytes except for the IFN-γ MΦ subset where it is detected at a higher relative rate. By contrast, *PADI*4 gene expression is suppressed in all MΦ subsets except the IFN-γ MΦ where *PADI*4 mRNAs are weakly detected. Consistently with mRNA analysis, PAD2 is detected in all polarised MΦ and more strongly expressed in IFN-γ MΦ whereas PAD4 is no longer detectable except faintly for the IFN-γ MΦ.
Conclusions This study shows that PAD2 is expressed at various degrees in the four analysed MΦ phenotypes but PAD4, only in the IFN-γ MΦ, both enzymes being expressed in monocytes. This reinforces the hypothesis of a role for monocytes and MΦ in genesis of ACPA epitopes in the ST.

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P006 ANTI-RA33 (HNRNP-A2/B1) AUTOANTIBODIES ARE ASSOCIATED WITH THE THERAPEUTIC RESPONSE TO METHOTREXATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction Besides the determination of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), anti-RA33 antibodies (which are directed to the nuclear antigen hnRNP-A2) could be of additional diagnostic and/or prognostic value in patients with rheumatoid arthritis (RA) because they are also found in RF/ACPA negative patients.[1]
Objectives So far, published data on anti-RA33 antibodies refer only to the IgG isotype.[2] It was therefore the aim of this study to measure the prevalence of anti-RA33 IgG, IgM and IgA antibodies in patients with RA and to determine their potential prognostic value regarding prediction of response to treatment.
Methods To determine the diagnostic sensitivity and specificity of anti-RA33 subtypes sera from 255 RA patients, 258 disease controls and 100 healthy subjects were tested by a prototype anti-RA33 EliA (Thermo Fisher Scientific) for the presence of anti-RA33 IgG, IgA and IgM antibodies. ACPA and RF were routinely measured by EliA and nephelometry, respectively. All RA patients had initially been treated with conventional synthetic drugs (mostly methotrexate, MTX) and were subsequently treated with at least one TNF inhibitor (TNFi). Therapeutic responses to MTX and TNF blocking biologicals were analysed in an inception cohort (n=104) who had started their DMARD therapy at our clinic. To define therapeutic responses the simplified disease activity index (SDAI) and American College of Rheumatology (ACR) responses were calculated.
Results Diagnostic specificity was >96% for all 3 anti-RA33 subtypes. Among the 255 RA patients, 11% tested positive for anti-RA33 IgG antibodies, 15% for IgM antibodies and 6% for IgA antibodies. Altogether, 62 patients (24%) had at least one type of anti-RA33 antibody: among these, 24 patients were RF-negative, 26 were ACPA-negative and 18 were RF/ACPA double negative. Thus, in 32 patients (13%), anti-RA33 was the only antibody specificity. Regarding responses to MTX therapy, the percentage of SDAI50 or ACR20 responders, respectively, was significantly higher (p=0.034 for SDAI50 and p=0.005 for ACR20) among anti-RA33 positive patients (with or without RF/ACPA) compared to anti-RA33 negative (but RF/ACPA positive) patients and RF/ACPA negative patients. Thus, 60% of the anti-RA33 positive patients as compared to 37% anti-RA33 negative patients showed a SDAI50 response; similar values were seen for ACR20 responses.
Conclusions Apart from their added diagnostic value anti-RA33 antibodies may have also prognostic value for prediction of therapeutic responses to MTX treatment. Therefore anti-RA33 antibodies may be taken into consideration as additional markers that might become helpful tools in therapeutic decision making.

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P007 IN RA, BECOMING SERONEGATIVE OVER THE 1ST YEAR OF DMARD TREATMENT DOES NOT TRANSLATE TO BETTER CHANCES OF DRUG-FREE REMISSION IN THE LONG-TERM

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Introduction Patients with RA harbour autoantibodies of various isotypes and directed against several post-translational modifications. Baseline seropositivity is a poor prognostic factor for sustained drug-free remission (SDFR). However, autoantibody levels may change and patients may become seronegative under treatment. It is unknown how often this happens, and whether decreasing levels or becoming seronegative early in disease, indicating disappearance of serological autoimmunity, improves chances of SDFR.
Objectives To longitudinally characterise the levels and presence of autoantibodies in RA patients and to investigate